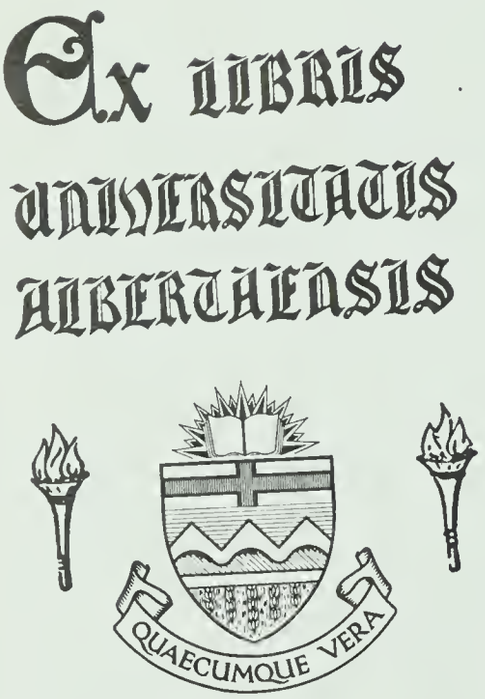

THE UNIVERSITY OF ALBERTA

MAMMALIAN SOMATIC CELL HYBRIDIZATION

BY

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Mammalian Somatic Cell Hybridization," submitted by Patrica Anne Millman in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Somatic cell hybridization studies have generally been concerned with the use of the somatic cell hybrid as a tool for genetic analysis. Few attempts have been made to determine whether this phenomenon of cell hybridization is simply an event induced by man-made techniques or whether it is in fact a basic underlying mechanism involved in the growth and evolution of some or all cell populations. The observation has been made that an increase in cell ploidy is often associated with the transformation of a primary cell culture to a permanent cell line, and with the occurrence of neoplasia, and subsequent growth and evolution of neoplastic cell populations. The possibility that a relationship exists between these increases in cell ploidy, and somatic cell hybridization has been suggested.

The present investigation was the first step in a larger project, the purpose of which is to determine whether somatic cell hybridization can occur *in vivo* in a solid neoplasm composed of two different neoplastic cell populations and if so, to determine whether this process of hybridization is involved in the subsequent evolution of such a "mixed" tumor. Before embarking on the *in vivo* studies it was considered necessary to provide evidence that somatic cell hybridization between the two choosen lines occurred *in vitro*. Obtaining this evidence was the purpose of the work reported here.

In this experiment somatic cell hybridization has been shown

to occur at a low frequency in a mixed *in vitro* culture of the Sarcoma 180 (Foley) murine cell line and the L-5178Y lymphoma murine cell line. Pilot studies have also been completed which indicate that both cell lines can be grown as solid tumors in C3H mice. Hence the proposed *in vivo* experiment in which the possible hybridization of these lines will be observed in a mixed solid tumor is, in fact, feasible.

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ACKNOWLEDGEMENTS

The scientific rewards of these past twelve months of research are recorded in the pages that follow. It would require at least as many pages to adequately acknowledge the more personal rewards which I have enjoyed through my association with the others involved in this project. My sincere thanks is extended to Dr. O. G. Thurston and to Dr. J. Weijer, my supervisors, and to Mrs. Maureen Huggins, my co-worker, for their invaluable assistance and encouragement.

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INTRODUCTION

The topic of this thesis is cell hybridization - specifically mammalian somatic cell hybridization. Hybridization of this type can be defined as genetic interchange between individual somatic units.[1] Until recently, somatic cell hybridization studies have been oriented toward basic genetics.[2,3] This writer's interest however lies in the relationship between cell hybridization and the initial formation and subsequent evolution of neoplastic cells. This study is concerned with the mechanisms by which heteroploid cells (specifically heteroploid cells with an increased chromosome number) appear in cell populations. As will be outlined later an increase in chromosome number is frequently associated with the transformation of a primary culture to a permanent cell line, and the change of a normal cell population to a neoplastic cell population.

REVIEW OF THE LITERATURE

History of Somatic Cell Hybridization Studies

The original work on cell hybridization was carried out by Barski[4,5] and co-workers in France in the year 1960. In his experiment he used two neoplastic cell lines of fibroblasts from mice - the high-cancer N1 line derived from clone NCTC 2472 and the low-cancer N2 line derived from clone NCTC 2555. Both these lines were heteroploid with chromosomal modal numbers of 55 and 62, respectively. Each line had distinctive features and could readily be distinguished from the other line: 1) The karyotype of each line was different. The karyotype of N1 contained only telocentric chromosomes as in the normal mouse cell, with one exception; One of the telocentric chromosomes was extremely long and hence distinctive. It was therefore a marker chromosome. The karyotype of N2 also contained many telocentric chromosomes but in addition each cell had approximately 13 metacentric chromosomes. These 13 metacentric chromosomes served to identify the presence of this cell line. 2) Each cell line also exhibited a difference in cell morphology and a difference in tumorigenicity which was expressed by the percentage of takes and the rapidity of tumor growth following innoculation into mice of the C3H strain.

Barski placed both these cell lines together in a culture flask containing Morgan's Synthetic Mixture 199 with 15 per cent horse serum in which both lines could proliferate. As the culture

was growing he watched the growth pattern for any change (both cell lines grew in a monolayer on the glass surface) and he did regular chromosomal analyses of the mixed culture. At about the third month after initiation of the culture, hybrid cells began to appear. By the end of the eighth month the hybrid cells made up about one-third of the total cell population. The hybrid cells showed a cell morphology that had characteristics in common with both parental lines. The karyotype of the hybrid showed a total of approximately 115 chromosomes, very near the sum of the modal numbers for the original cell lines. The number of metacentric chromosomes present was approximately 12, which indicated that only one cell from N2 was present. The marker chromosome from N1 was present in many hybrid cells. The hybrid cells expressed the high tumorigenicity characteristic of the N1 line when re-innoculated into C3H mice.

Barski suggested a two-stage process by which somatic cell hybridization might come about: a) Cytoplasmic fusion and subsequent inclusion of two or more nuclei of different origin into one cell. b) Simultaneous mitosis of the two or more different nuclei resulting in an association of the chromosomal complements in a newly formed nucleus.

This work by Barski was supported by the research done in <u>in vitro</u> cultures by subsequent workers.[6,7,8,9] Within the next few years many hybrid cultures originating from a variety of parental

lines had been produced.[10,11] In some cases the hybrid cells dominated the cultures and completely overgrew the parental lines. In other experiments they appeared not to have a selective advantage and remained only at very low frequencies in the culture.[7] It was noted that in some cases a change in environmental conditions, for example, a decrease in temperature could determine whether or not the hybrids obtained a selective advantage.[12] As the work accumulated, it became evident that somatic cell hybridization was a very generalized phenomenon because it could occur between a great variety of different cell lines. It was not a mechanism limited by the species of origin of the material. The early experiments were performed between two cell lines of murine origin but since then hybrid cells have originated from crosses involving mouse and rat[13], mouse and hamster[14,15], human and mouse[16], and cattle and mink[17] combinations. There was also no barrier between the union of cells from neoplastic tissues with cells from non-neoplastic tissues[12] or between diploid and heteroploid cells[18], or between diploid cells of two different origins[17] or between primary cells and cells from permanent cell lines.[13] These hybrid cells appeared to be capable of indefinite serial propagation.

Hybrid cells were identified earlier in subsequent cultures than they had been in Barski's original culture - some appeared within as short a period of time as 24 hours.[19] Many people who were working with hybrid cells that did not completely overgrow the culture, cloned the cells.[20] In this way they could study in detail the properties

of the hybrids and also study their evolution as they grew in tissue culture. The majority of hybrids were unable to retain the complete number of chromosomes with which they originated - apparently the large number of chromosomes hampered the mitotic mechanism or perhaps the mitotic rate of the two chromosome complements within the one nucleus was not completely syncronized. The loss of chromosomes, which can be referred to as segregation, appeared to be random in some cases and preferential to one parent in others.[14, 16, 18]

Soon after the original experiments by Barski, Harris and his associates[21] began experimenting with mixed cultures of cells to which viruses inactivated by ultra violet light had been added. These inactivated viruses caused fusion of cells from each cell line to form multinucleate cells - or artificial heterokaryons. Harris found that some of the nuclei of heterokaryons underwent mitosis and those nuclei which entered mitosis synchronously usually fused together. The mononucleate hybrids that were the result of fusion of a large number of single cells did not appear capable of continued multiplication, but mononucleate cells containing one chromosomal set from each parent were still found to be undergoing mitosis many days after cell fusion. Harris used combinations of Hela cells (of human origin) and Ehrlich ascites cells (of mouse origin) to produce the hybrid cells.[22,23] He also used Hela cells in combination with cells from rats, rabbits and chickens.[24]

Harris[24] suggested that the hybrid cells found in Barski's

experiment were probably also the result of viral action - perhaps all cell hybridization was due to the presence of viruses carried by the cell lines in latent form. This was indeed a possibility but attempts have been made to show the presence of viruses in the cultures of workers professing to have produced hybrids spontaneously - no viruses have been detected.[19] It was most likely that hybridization could occur spontaneously and if a virus was present, the clumping effect provided by the virus sped up the process and made it more effective.[25, 83]

In 1964, Littlefield[26,27] introduced a method for the rapid detection of hybrid cells in a culture. This selection technique was based upon enzyme deficiencies correlated with drug resistances in two clonal lines of L cells. Clone A3-1 was resistant to 8-azaguanine. This resistance was the result of the absence of the enzyme guanylic acid - inosinic acid pyrophosphorylase in the A3-1 clone. Clone B34 was resistant to 5-bromodeoxyuridine. This resistance was due to the absence of the enzyme thymidine kinase. If hypoxanthine, aminopterin, and thymidine were added to the medium in which the cells were growing then the endogenous biosynthesis of purines and thymidylic acid was blocked with aminopterin. Cells deficient in either pyrophyosphorylase or kinase did not survive because they could not utilize hypoxanthine or thymidine present in the medium. While these lines were deficient in either pyrophosplorylase or kinase, each contained the other enzyme. If one cell of each type fused together, the resulting hybrid cell would contain some of both

enzymes and would survive exposure to aminopterin.

The mixed cultures were initiated in normal media - this gave the hybridization process a chance to begin - then all the cells were removed from this medium and placed in the selective medium. Neither of the parental lines survived in the selective medium, but hybrids that had been formed had both enzymes present and hence were able to grow well in the selective medium. The resulting culture was then composed entirely of hybrid cells.

Davidson and Ephrussi[28] modified Littlefield's method slightly. They showed that if cells carrying one of the biochemical markers were crossed with slow growing, unmarked diploid cells, morphologically distinguishable multilayered hybrid colonies appeared on a monolayered background formed by the contact inhibited diploid parent. The hybrid cells were then isolated by mechanical scrapping with a micropipette. Since both the methods of Littlefield and Davidson and Ephrussi detected even the smallest number of hybrids in a culture, they were used extensively by many workers.[2 3,13,14,16,18,29,30]

Transitions in Chromosome Number from the Diploid to the Heteroploid State

Previous studies[32,33,34,36,37,38,41,48,50] have indicated that transitions in chromosome number from the diploid to the heteroploid state were associated with: 1) The transformation of primary cultures to permanent cell lines in vitro. 2) Carcinogenesis and subsequent changes in the progressive growth of a tumor.

Normal mammalian tissue, except for a small proportion

of polyploid cells, was found to have a chromosome number precisely defined at the diploid level.[31,32,33,34] Malignant tumors and permanent cell lines both originated from normal diploid tissue, yet their karyotypes presented a striking contrast to the stable diploid patterns of normal cells. The large majority of tumors and permanent cell lines had a heteroploid modal chromosome number, with appreciable fluctuation around the mode.[40]

The mechanism of transformation of a primary diploid culture to a permanent cell line was not understood. The cultures passed through three stages: (a) an interval of progressive growth, (b) a dormant period during which the majority of primary cultures died and (c) a transformation which involved the emergence of a variant cell type that was able to grow as a permanent cell line.[39] This variant cell type differed in many ways from the normal diploid cell type from which it arose. The variant cell displayed a change in growth pattern, cell morphology and cell metabolism. In addition dedifferentiation of cell function, a change in chromosome structure and in the majority of cases a shift from the diploid to the heteroploid chromosome number occurred.[32 34,39 47 48 49 50] Heteroploidy was not completely diagnostic for permanent cell lines - occasional permanent lines were diploid or pseudodiploid.[42]

The shift to heteroploidy in long term in vitro cultures appeared to involve cells with variant chromosome numbers that had

arisen after explantation of the normal tissue.[43] These aneuploid cells grew more rapidly at the time of heteroploid shift than did the euploid cells.[44] The apparent selective advantage of the variant cells may have been the result of an adaptive adjustment by the primary population to the conditions of *in vitro* culture.[1] The same apparently adaptive adjustment was observed in cell populations explanted directly from tumors. In this case the primary cultures were heteroploid to begin with but they underwent even more drastic changes in karyotype before a permanent line was established.[45 46] Once a permanent line was established variation in chromosome number proved to be a continuing process - even clonal populations derived from single cells showed a fluctuation in chromosome number.[40]

The process of carcinogenesis also involved a change in cell type. Neoplastic cells exhibited dedifferentiation of cell function, a change in cell morphology and growth pattern, structural alterations of the chromosomes and in most cases a shift from a diploid to a heteroploid chromosome number.[32 33,34 36 37 38,41] Once formed malignant tumors were capable of further karyotypic change and hence greater degrees of heteroploidy as their growth progressed. Again as in the case of permanent cell lines, not all tumors had a heteroploid chromosome number, there were a few exceptions.[37 51 52]

The shift to heteroploidy in permanent cell lines and tumors led some investigators to equate the origins of both with the process of neoplastic conversion.[53] This view found support in numerous

studies which showed that neoplastic transformation can take place in vitro. With mouse cells it appeared that most, if not all, permanent populations eventually acquired tumor-producing properties.[54] However the initial transition from normal to neoplastic states could not be directly correlated with the appearance of heteroploidy since; (1) many heteroploid permanent cell lines showed no neoplastic properties whatsoever and (2) some frankly malignant tumors showed no immediate shift in chromosome pattern.[34,37,51,55]

The lack of direct correlation between acquisition of neoplastic properties and a heteroploid shift in chromosome number did not rule out the possibility that a heteroploid shift may be one of a number of events that serve to trigger neoplastic transformations or that a heteroploid shift may reinforce and aid in the progression of neoplastic transformation by providing genetic implementation for variability initiated by another cause.[56]

It was suggested[57] that the shift to a heteroploid chromosome number in tumors and in permanent cell lines might follow two possible pathways: 1) The chromosome number may have become aneuploid by the loss or gain of one or two chromosomes, and then by some mechanism, the chromosome number was doubled. The resulting cell line would have a hypotetraploid or hypertetraploid chromosome number, depending on whether chromosomes had been lost or gained before doubling; or, 2) The diploid cell may have become tetraploid by any one of a number of possible mechanisms. A subsequent loss of chromosomes would then

have resulted in a hypotetraploid or hypertriploid chromosome number.

Ruddle[58] showed that cell populations derived from pig kidney originated as diploid cells, then became tetraploid, and subsequently lost chromosomes to become hypotetraploid and hypertriploid. He also noted that in this particular case the cycle of change began again. Polyploids of a new order appeared by chromosome doubling within the heteroploid stem cells and subsequent diversification took place once more at the higher level.

The appearance of heteroploid cells as the dominant components in permanent cell lines and in tumors was explained by assuming that these variants possessed favourable growth characteristics. It was considered significant that the karyotypic shifts (in permanent cell lines) coincided with progressive slowing of population growth and decline in cell number to a low level.[39,59] This critical stage may have been produced by a slow but progressive breakdown and loss of key cellular components which were not subsequently replaced at a rate equal to cell multiplication. Since cell volume was found to be a function of ploidy,[60] the larger polyploid cells with a more favourable surface-volume relationship would be expected to show some kind of selective advantage. The polyploid state appeared to act as a buffer against the occurrence of structural rearrangements in the karyotype - the increased gene dosage compensated for imbalance or specific deficiency due to any single chromosome loss. Karyotypic remodeling in the polyploid population may then have resulted in the

growth of variant cell lines each with a heteroploid number. These heteroploid cell lines may have had properties more favourable to growth and proliferation under the existing conditions and hence they overgrew the normal diploid population.

From the preceding discussion the importance of mechanisms whereby cells may shift from diploid to heteroploid states (particularly the hypotetraploid and hypertriploid states) becomes apparent. Many possible mechanisms have been discussed by other authors concerning the origin of heteroploid cells with increased chromosome numbers. Most investigators have suggested and provided evidence for mechanisms originating with a single uninucleate cell. For example (a) endreduplication or endomitosis (b) c - mitosis (c) failure of the cytokinesis to occur and (d) teloreduplication are all processes which take place in one uninucleate cell and result in the production of a heteroploid uninucleate cell with an increased chromosome number[61,62,69,70]. Binucleate cells may be an intermediate stage in processes (c) and (d).

A few investigators have considered the process of cytoplasmic fusion and subsequent nuclear fusion of two cells (probably synonomous with somatic cell hybridization) as a possible mechanism by which the diploid - heteroploid shift occurs.[21,68,71] This process would probably involve the formation of a binucleate cell as an intermediate step. Observations by several workers appear to confirm that fusion of nuclei in a binucleate cell does occur and this fusion results in the production of a uninucleate cell with a heteroploid chromosome

number. The nuclear fusion can take place, (1) when both nuclei are in interphase[21] (result - one hetroploid cell with an increased chromosome number) or (2) when both nuclei enter mitosis synchronously[21,63,64,60,71] (result - two heteroploid cells or one cell with two heteroploid nuclei). Direct evidence that cytoplasmic fusion takes place is more difficult to obtain, and to date, all the evidence for cytoplasmic fusion is indirect. Harris[21] observed cell clumping and subsequent cytoplasmic and nuclear fusion of cells in a culture to which viruses had been added. Roizman[68] made similar observations on another cell culture to which the same addition had been made. He also pointed out that viruses were only one part of a diverse group of agents which induced cytoplasmic and nuclear fusion, (that group also includes bacteria, parasites, fungi, silica, sutures, cellophane, oils, fats, cholesterol and parathyroid hormone).

There may or may not be a correlation between the presence of binucleate cells and the incidence of cell fusion (hybridization), but it is interesting to note that in several cases an increase in the number of binucleate cells in a cell population has been observed when that cell population has been under some kind of "environmental stress" or change. For example an increase in the number of binucleate cells has occured when a cell population has been subjected to the following: (1) stress due to radiation damage[65,66,67] (in this case amitosis may be involved in the production of bincleate cells - DNA content studies would be necessary for conformation); (2) the change due to the presence of viruses in a cell population;[21,68] (3) the stress produced by extreme starvation in a cell population[69]; and (4) the stress resulting from death of the organism.[72]

Other researchers have suggested that the production of heteroploid cells by the fusion of cells (somatic cell hybridization) may be a mechanism significant in the progressive changes that occur in neoplastic cell populations. In 1967 Defendi and co-workers[73] initiated a study of hybridization in a mixed cultures of neoplastic and normal cells in hopes that the hybrids formed might yield some information as to the nature of changes involved in carcinogenesis. They wanted to determine the tumorigencity and antigen make-up of hybrid lines obtained by crossing polyoma-transformed mouse cells with normal diploid mouse cells. The polyoma-transformed cells were neoplastic and possessed the polyoma-induced transplantation and complement-fixing antigens. They had a hypotetraploid modal chromosome number and approximately seven biarmed chromosomes present in each cell. The normal diploid mouse cells were taken from a strain of mice carrying the T-6 translocation and hence the two T-6 translocation chromosomes served as markers in these cells. The resulting hybrids from these two cell lines showed a dominance of the characteristics from the neoplastic line. Most important, however, is that in most hybrids the tumorigenic properties were greater than that of the original parental line. There were several possible explanation for this enhancement; (1) Defendi's results suggested that polyoma-transformed lines were heterogenous populations comprising cells of different degrees of tumorgenicity. Because of different properties of their cell surface, the more tumorigenic cells may have fused more readily with normal cells than the less tumorogenic ones. On the other hand, (2) the frequency of

fusion of all cells of the polyoma-transformed line with normal cells may have been the same but the hybrids resulting from the fusion of the more neoplastic cells may have had a selective advantage over the other components of the hybrid populations propagated *in vitro*.

Whatever the mechanism involved, the enhancement of tumorigenicity of the parental cells following hybridization with normal cells, leads to the question: Is the fusion of tumor cells with adjacent normal cells a possible pathway for tumor progression in vivo?

In 1968 Agnish and Fedoroff[74] published a paper which supports the idea that somatic cell hybridization does in fact take place *in vivo*. They were studying different cell types found in two sublines of Ehrlich ascites tumor. They distinguished three cell types: (a) small cells with purple staining nuclei (mode 75 chromosomes) (b) medium sized cells (mode 110 chromosomes) (c) vacuolated cells. They also found a fourth type (small cells with dark staining nuclei) among the tumor cells - these were believed to be host lymphocytes. Evidence that Agnish and Fedoroff had gathered previously, indicated the medium sized cells and the vacuolated cells were the same cells in different functional states. The original purpose of the work was to determine the origin of the vacuolated cells. However, Agnish and Fedoroff made an interesting observation - there was an increase in the number of small cells with dark staining nuclei (supposed host lymphocytes) in a 24 hour ascites tumor initiated by the injection of small cells with purple staining nuclei. The possibility occurred

to them that these host lymphocytes might be of possible significance in terms of the progression of the tumor. Possibly hybridization occurred between the small cells (75 chromosomes) and the lymphocytes (40 chromosomes) followed by a partial segregation and rearrangement of their chromosomes to form the medium sized cells with modal number of 110. If this in fact occurred, it would be a good example of somatic cell hybridization between tumor cells and normal host cells in vivo.

Agnish and Fedoroff presented two lines of evidence that appear to support the idea, that hybrids are being formed in vivo: (1) Ascites tumors were initiated by the innoculation of a mixture of radioactively labelled tumor cells and unlabelled bone marrow cells. After 16 hours a small percentage of the cells were partially labelled, indicating possible hybrids. (2) Bone marrow cells of mice with distinct chromosomes and acentric fragments induced by radiation treatments were mixed with cells from the tumor fraction containing mostly small cells with purple staining nuclei and the mixture was used to initiate ascites tumors. Upon chromosomal analysis of these tumors, cells were found with chromosome numbers in the pentaploid range, showing one or more of the aberrations induced by radiation.

The evidence was indirect but it suggested that hybridization had taken place between the host cells and the small tumor cells in vivo.

OBJECTIVES

The research work to be reported here is the first part of a project having the following objectives:

(a) to produce hybrid cells *in vitro* from two malignant cell lines with distinctive karyotypes.

(b) to establish the two malignant cell lines as transplanted solid tumors *in vivo*.

(c) to produce hybrid cells *in vivo* in a mixed solid tumor from the above two malignant cell lines.

(d) to examine the possibility that hybrid cells produced *in vivo* in a solid tumor may aid in the evolution and progressive growth of that tumor.

MATERIALS AND METHODS

A decision was made to use mouse cell lines in the following experiments because of: (a) The availability of a variety of mouse cell lines (b) The previous hybridization successes reported in the literature with mouse cell lines.

From five available murine cell lines (two ascites tumors and three neoplastic permanent cell lines) two cell lines were chosen with compatible growth characteristics and distinctive karyotypes.

Cell Lines (available)

Ehrlich ascites carcinoma (EAC) and Ehrlich ascites carcinoma R2 (EAC-R2)

The EAC tumor line, and the EAC-R2 subline were kindly supplied by Dr. A.R.P. Paterson (University of Alberta Cancer Research Unit, McEachern Laboratory). The origin of the EAC-R2 subline has been described by Caldwell, Henderson and Paterson.[75] The chemical difference between the two tumors lay in the resistance of EAC-R2 to the growth-inhibitory effects of 6 - (methylmercapto) purine ribonucleoside. The tumor lines were not grown in tissue culture, but were studied directly upon extraction from the peritoneal cavity of mice.

Strain L clone 929 (L-929)

Strain L cells were originally derived by Earle[76] from connective tissue of a C3H mouse, and Strain L clone 929 was established (by the capillary technique for single cell isolation)

from the 95th subculture generation of Strain L.[77] The Strain L clone 929 cells analysed in this experiment came from two different sources. One group of cells which had been obtained originally from the American Type Culture Collection(1 was kindly supplied by Dr. A.R.P. Paterson. The other group was purchased directly from Microbiological Associates(2. It was possible, since these two groups of cells had been maintained separately that some karyotypic variation might have occured which would allow one group of the L-929 cells to be identified in the presence of the other. Both groups of cells grew in a monolayer in Minimum Essential Medium supplemented with 15 per cent bovine serum.

L-5178Y mouse lymphoma cell line

The L-5178Y lymphoma cell line was obtained from Dr. A.C. Sartorelli of Yale University through the courtesy of Dr. A.R.P. Paterson. The line had been maintained in male BDF_1 mice by intraperitoneal transplanatation of 10^7 tumor cells each week. In vitro, L-5178Y cells grew in suspension, and in spinner culture in Fischer's Medium for Leukemic Cells of Mice supplemented with 15 per cent horse serum.

(1 American Type Culture Collection
12301 Parklawn Drive
Rockville, Maryland 20852

(2 Microbiological Associates, Inc.
4733 Bethesda Avenue
Washington, D.C.

Sarcoma-180 (Foley) cell line (S-180)

The Sarcoma-180 (Foley) cell line was established by G.E. Foley et al in 1959 from an S-180 sarcoma maintained in adult CFW mice.[78] The S-180 (Foley) cells used in this experiment were purchased from Microbiological Associates Inc.[2] They normally grew in a monolayer in Minimum Essential Medium supplemented with 15 per cent horse serum. The cells from this line could also be grown in a spinner flask in Fischer's medium for a period of four to six weeks.

In the course of the study it became apparent that the growth characteristics and karyotypes of L-5178Y mouse lymphoblast and Sarcoma-180 (Foley) made them a suitable pair of cell lines for hybridization experiments (see details in Results).

Growth in Mixed culture

To enhance the possibility of contact between cells of the two different cell lines, the mixed culture was maintained in a spinner flask.[69] In this apparatus, the two cell populations were kept in uniform distribution in the medium by a magnetic stirrer which activated a teflon-coated bar mounted on a swivel within the culture vessel. Figure 1 is a photograph of one of the spinner flasks used in this experiment.

The mixed culture was initiated by the innoculation of 3×10^6 cells each of the S-180 and L-5178Y cell lines into a 500 ml.

spinner flask containing 150 ml. of Fischer's medium supplemented with 15 per cent horse serum; with penicillin and streptomycin in a final concentration of 100 u. and 100 mcg. per ml. of medium respectively. The medium was removed every third day and replaced with fresh medium. The progress of the mixed culture was followed by chromosomal analysis performed at least once, and sometimes twice weekly. Preparations were also made of the intact cells to determine the presence of binucleate cells.

Separate cultures of the S-180 and the L-5178Y cell lines were maintained as controls. Their treatment and analysis was identical to that of the mixed culture.

Chromosomal analysis

Metaphase spreads suitable for chromosomal analysis were prepared from the mixed culture of S-180 and L-5178Y in the following manner. Approximately 16 hours before harvesting one and one half ml. of colcemide solution(3 were added to the culture medium. After 16 hours 50 ml. of this mixture were poured directly into a centrifuge tube and spun at 800 r.p.m. for 10 minutes. This procedure resulted in approximately 0.5 ml. of packed cells being recovered in the

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(3 One mg. Colcemide in 12.5 c.c. distilled water (CIBA Company, Dorval, Quebec)

centrifuge tube. The supernatant was discarded and hypotonic salt solution(4 was slowly added to the packed cells. The cells were resuspended in the hypotonic solution and left for 30 minutes. One ml. of Hyaluronidase solution(5 was then added to the resuspended cells (to separate individual cells). The cells were spun down for five minutes at 800 r.p.m. The supernatant was discarded. Slowly and without disturbing the packed cells, fresh glacial acetic acid-methanol fixative(6 was added to a maximum of 20 ml. of fixative. The tube was corked tightly and refrigerated at 4°C. for 30 minutes. Following refrigeration, the cells were spun down at 800 r.p.m. for five minutes. The fixative was changed a minimum of three times. After fixation, a test slide was prepared by dropping a small amount of cell suspension from a pipette onto a slide and then passing the slide through a flame to ignite the fixative present in the suspension. If satisfactory chromosome spreading did not occur at this point, the fixative was changed several times more. Permanent slides with suitable metaphase spreads were prepared in the same manner in which the test slide had been prepared. These slides were stained in acetic orcein for one hour. Slides with insufficiently stained spreads were placed in cresyl violet for 10 minutes. Photomicrographs were produced from the slides by the standard method.

(4 One ml. Hank's Balanced Salt Solution in 4 ml. of distilled water.

(5 0.05 gm. Ovine Testes Hyaluronidase (Sigma Chemical Company, St. Louis, Mo. 63118) in sodium hypophosphate - citric acid buffer, pH 5.3.

6) One part glacial acetic acid: three part methanol.

Preparation of Intact Cells

Slides suitable for examination of the cultures for binucleate cells were prepared in the following manner by omitting the colcemide, hypotonic saline and hyaluronidase steps and staining with hematoxylin and eosin instead of acetic orcein. Approximately 50 ml. of medium and cell mixture were removed from the spinner flask, placed in the centrifuge tube and spun at 800 r.p.m. for 10 minutes. The supernatant was removed. Slowly and without disturbing the packed cells, fresh glacial acetic acid-methanol fixative was added to a maximum of 20 ml. of fixative. The tube was corked tightly and refrigerated at 4°C for 30 minutes. Following refrigeration, the cells were spun down at 800 r.p.m. for five minutes. All but 5 ml. of the fixative was removed and the packed cell volume was gently resuspended in the remaining fixative. Every attempt was made to handle the cell suspension gently in order to prevent rupturing of the cell membranes. A small portion of the cell suspension was then placed on a slide and allowed to air dry. The dry slides were then placed in hematoxylin and eosin stain, coverslipped and examined for the presence of binucleate cells.

Establishment of solid tumors in C3H mice

Innoculations of 10^4, 10^7 and 10^{10} cells from S-180 and L-5178Y cell lines were made intramuscularly into the thigh of mice of the C3H strain[2] in order to determine if each cell line would produce a solid tumor, and to determine cell innoculum required in each case.

RESULTS

Karyotype Studies

Ehrlich ascites carcinoma (EAC) and Ehrlich ascites carcinoma R2 (EAC-R2)

Chromosomal analysis did not reveal any morphologic differences between the chromosomal complements of the EAC and the EAC-R2 ascites tumors. The chromosome number for both tumors was in the hypotetraploid range for the normal mouse cell. The chromosomes were all telocentric and acrocentric except for two metacentric chromosomes, one large and one medium sized. Figure 2 shows a representative karyotype of the EAC line. (72 chromosomes)

Strain L clone 929 cell line (L-929)

Chromosomal analysis indicated the karyotypes of the two groups of L-929 cells were similar. Each had a hypertriploid chromosome number. Approximately one-third of each karyotype was made up of metacentric chromosomes, the remaining portion was composed entirely of acrocentric or telocentric chromosomes. Both groups possessed the same marker chromosome. This marker had two alternative forms generally referred to as the T and the D forms.[80] The two alternative forms were present in different proportions in each group of L-929 cells. In one group the proportions were D=41%, T=59% and in the other group they were D=65%, T=35%. The D form chromosome was a long submetacentric chromosome with a deep secondary constriction in the long arm. This chromosome could be roughly divided into three sections: The short arm,

the middle piece which was approximately twice as long as the short arm, and the end piece located distal to the secondary constriction. The middle piece could be subdivided into three sections by two less conspicuous secondary constrictions. The T form chromosome was a long metacentric chromosome with a deep secondary constriction on each arm. It has been suggested that the T form is a derivative of the D form because each arm of the T is morphologically identical with the long arm of the D. It is possible that the D chromosome at first lost its short arm and later by misdivision of the centromere became an isochromosome. Figure 3 shows the D marker in a cell of the L-929 line (67 chromosomes) and Figure 4 shows that T marker in another cell of the L-929 line (66 chromosomes).

L-5178Y mouse lymphoma cell line (L-1578Y)

Chromosomal analysis of the L-5178Y cell line indicated a bimodal distribution of the chromosome number. In 70 of 100 cells counted the chromosome number was close to the stemline (s) number of 42 chromosomes. In 30 of 100 cells the chromosome number was close to the double stemline (2s) numbers of 81-82 chromosomes. Figure 5 is a histogram showing the number of chromosomes per cell in 100 cells of L-5178Y. There were no marker chromosomes present. The whole complement was composed of acrocentric and telocentric chromosomes. Figure 6 shows a representative karyotype of a stemline L-5178Y cell. (41 chromosomes)

Sarcoma 180 (Foley) cell line (S-180)

Chromosome analysis of the S-180 cell line revealed a cell population composed of a stemline (s) group (92 of 100 cells) and a double stemline (2s) group (eight of 100 cells). The chromosome counts for 100 stemline cells ranged from 80-90 with a mode at 87 (Figure 7). The double stemline cells had chromosome numbers in the tetraploid and hypotetraploid range. The karyotype was composed of acrocentric and telocentric chromosomes except for three distinctive metacentric chromosomes (one large, one medium sized, and one small) and, on the average, two minute chromosomes. Occasional pairing of one of the metacentric chromosomes was noted. Figure 8 shows a representative karyotype of a stemline S-180 cell (84 chromosomes).

There are no metacentric chromosomes in a normal mouse karyotype. The karyotype is made up entirely of telocentric and acrocentric chromosomes. However, the finding of metacentric chromosomes in the karyotype of a permanent mouse cell line is a frequent occurence.[1] The origin of these metacentric chromosomes may be the result of any one of several mechanisms.[57] In the S-180 cell line there are metacentric and minute chromosomes present in each cell. The presence of both types of chromosomes together suggests that they may have originated from a reciprocal translocation between two chromosomes - an acrocentric chromosome and a telocentric chromosome. If the acrocentric chromosome broke beyond the centromere and the telocentric chromosome below the centromere, followed by a reciprocal exchange of material, and subsequent fusion, the result would be the formation of a metacentric chromosome and a minute chromosome. (Figure 9)

Once the karyotype and the growth requirements for each cell line had been established, the characteristics of each line were considered to determine the best possible combination that could be used in a mixed culture.

Ehrlich ascites carcinoma (EAC) and Ehrlich ascites carcinoma R2 (EAC-R2)

Because there were no obvious chromosomal differences between the karyotypes of the EAC cell line and the EAC-R2 subline, these lines could not be used together in a mixed culture. It was discovered that neither the EAC or the EAC-R2 lines would grow well *in vitro*, and hence it would have been technically difficult to combine either line with any of the other available cell lines *in vitro*. In retrospect, an opportunity may have been missed since an *in vivo* combination could have been made between either of the EAC or the EAC-R2 cell lines and the L-5178Y mouse lymphoma, because all these lines grow well in an ascitic form. At the time the author considered the hybridization of cells *in vitro* an important first step to the hybridization of the same cells *in vivo*.

Strain L clone 929 (L-929) and Sarcoma 180 (Foley) cell lines

The karyotypic difference between the two groups of L-929 cells was not sufficient to allow the identification of one group in the presence of the other and therefore, these cells were not a suitable combination for a mixed culture. A combination of the L-929

cell line with the S-180 (Foley) cell line appeared satisfactory. The karyotypes of each were distinctive and both cell lines could be grown in a monolayer in Minimum Essential Medium supplemented with horse serum and bovine serum. A mixed culture was initiated using these two lines and it is most likely that a small number of hybrid cells have been produced, the results, however, have not been studied in detail since the suspected number of hybrids is very low.

L-5178Y mouse lymphoma and Sarcoma-180 (Foley) cell lines

The S-180 and the L-5178Y cell lines appeared to be the ideal pair for growth in mixed culture. The karyotypes of the two lines were sufficiently distinctive to identify one cell line in the presence of the other, and although after a prolonged period of time there was deterioration of the S-180 cells, both cell lines grew equally well for at least four to six weeks in a spinner culture in Fischer's medium supplemented with 15 per cent horse serum. Growing the two cell lines in a spinner culture was considered necessary to ensure that cells of each line would be in direct contact with cells of the other line in the mixed culture. Normally the S-180 cell line grows in a monolayer and the L-5178Y cell line grows in suspension. If no mechanical mixing took place in the mixed culture, the S-180 cells would grow on the glass surface and the L-5178Y cells in suspension in the medium and there would be very little direct contact between the two.

Control cultures of the S-180 and the L-5178Y cell lines were maintained in separate spinner cultures under conditions identical to that of the proposed mixed culture. Chromosomal analysis of these cultures indicated that the percentage of double stemline (2s) cells present in the S-180 spinner culture was greater than the percentage of double stemline cells present in the monolayer cultures of the S-180 analysed at the same time. In the monolayer culture eight of 100 cells of the S-180 cell line were double stemline cells and in the spinner culture 34 of 100 cells were double stemline cells (Table I). This increase is highly significant ($X^2=20.4$ $P<0.005$). No such increase in the number of double stemline cells was noted in the L-5178Y spinner culture. As was indicated previously (Figure 5), the percentage of double stemline cells in this line was high (30 per cent) before the line was placed in the spinner culture.

Growth and Analysis of the Mixed Culture S-180 and L-5178Y

The growth rate of the mixed culture of L-5178Y and S-180 cells changed with time. Initially the cells grew rapidly and increased ten fold in number in each three day period. After approximately three weeks the growth rate began to decrease and by the end of five weeks the cells in the mixed culture had died. The experiment was repeated four times. In each case the culture was initiated with L-5178Y and S-180 cells in a 1:1 ratio and no new cells were added throughout the entire growth period. The mixed cultures survived for periods of

34 days, 36 days, 20 days, and 41 days respectively. Preparations for chromosomal analysis were made once or twice weekly. In almost all preparations stemline cells from both the S-180 and the L-5178Y cell lines could be identified and polyploid cells were noted in high frequency, but because of poor chromosome spreading the polyploid cells could not be accurately identified. Preparations satisfactory for detailed chromosomal analysis were obtained from the fourth mixed culture on day 22 and day 23 of its 41 day growth period. In order to eliminate incorrect classifications of mitotic figures in this analysis, only those figures which appeared complete and had satisfactory chromosome spreading were chosen. These criteria, although necessary, will influence the reported results, in that the proportion of lower ploidy cells present in the results will be exaggerated. The combined results of chromosomal analysis of the fourth mixed culture on day 22 and day 23 of its growth period are as follows: Two hundred metaphase cells were analyzed; 57 cells were s L-5178Y; 119 were s S-180; four were 2s L-5178Y; 11 were 2s S-180. Nine cells were identified as hybrid (L-5178Y + S-180) cells. Figure 10 is a histogram illustrating these results. All nine hybrid cells contained the metacentric marker chromosomes from the S-180 parent. Three of the hybrid cells had a chromosome number close to the number expected from the combination of an s S-180 cell with a 2s L-5178Y cell (87 + 81 = 168). The chromosome number in the remaining six hybrids approximated the number expected from the combination of an s S-180 cell with an s L-5178Y cell (87 + 42

= 129). Table II shows the chromosome counts for the nine observed hybrid cells. Figure 11 is a photograph of one of the hybrid cells. This cell has three metacentric marker chromosomes (large, medium-sized and small) and a chromosome number of 132 (s S-180 + s L-5178Y). Figure 12 is a photograph of another hybrid cell. This cell has four metacentric marker chromosomes (one large, one medium-sized and two small) and has a chromosome number of 168 (s S-180 + 2s L-5178Y).

Analysis of test mixture

To exclude the possibility that these hybrid cells had arisen merely by change superimposition of an S-180 cell and an L-5178Y cell which spread out together after fixation to give a single group of chromosomes, the following test was performed. Colcemide was administered to separate cultures of S-180 cells and L-5178Y cells for the same period of time that the mixed cultures had been exposed to the mitotic poison. Then an equal number of cells from each cell line were combined. This 1:1 mixture of S-180 and L-5178Y cells was treated immediately by the same method used to fix and stain the mixed cultures for chromosome analysis. The resulting mitotic figures were then analyzed using the same criteria that had been used for the mixed cultures. One hundred cells were analyzed; 42 were s S-180, 42 were s L-5178Y and 16 were 2s L-5178Y cells. There were no "hybrid" cells found. The 95 per cent confidence limits for the mixed culture are six and 13 (nine hybrid cells in 200 counted). The 95 per cent

confidence limits for the results from the test mixture are zero and four (no hybrid cells in 100 counted).[81]

Analysis for Binucleate Cells

Analysis of the slides prepared for the identification of binucleate cells provided the following information: Fourteen of 300 cells analyzed from the mixed (L-5178Y + S-180) culture were binucleate; seven of 300 cells analyzed from the separate cultures of L-5178Y and S-180 were binucleate. The difference between the number of binucleate cells present in the mixed culture and in the separate cultures, is not significant ($p > 0.10$) - Table III. Figure 15 is a photograph of a binucleate cell found in the mixed culture.

Growth of the Sarcoma-180 (Foley) and L-5178Y cell lines as solid tumors

A pilot project to determine the feasibility of growth as solid tumors of the S-180 and L-5178Y cell lines was carried out. Intramuscular innoculation of 10^4 cells from the S-180 cell line produced observable solid tumors in three to four days in 10 out of 10 C3H mice. Death due to tumor growth occurred within 20 days. Figure 14 shows a seven day old solid tumor of S-180 in the hind limb of a C3H mouse. Intramuscular innoculations of at least 10^{10} cells of the L-5178Y cell line were necessary to produce observable solid tumors in the C3H mice. These tumors appeared after three to

four days in 10 out of 10 of the mice injected but tumor regression occurred within 21 days in all of the mice. Subsequent massive injections of L-5178Y (10^{20} cells) have resulted in tumors which caused the death of the animal. Figure 13 shows a seven day old solid tumor of L-5178Y in the hind limb of a C3H mouse. It appears that an in vivo combination of these cell lines is feasible but further studies on in vivo growth of each cell line are necessary before hybridization experiments can be carried out.

DISCUSSION

The large number of chromosomes present in the hybrid cells and in the other polyploid cells found in the mixed culture, made exact chromosomal analysis difficult because the chromosomes in these cells were in the great majority of cases clumped and overlapping. As a result only "perfectly" spread and apparently complete cells were included in the reported results. As was mentioned before these criteria have necessarily influenced the reported results. The lower ploid cells (i.e. the stemline L-5178Y cells and stemline S-180 cells) have been recorded in the results in a higher proportion than they were actually present in the culture. The reason for this is that they had fewer chromosomes and hence a greater chance of producing "perfectly" spread and apparently complete mitotic figures.

It is the author's opinion that hybridization between the L-5178Y and S-180 cells occurred early in the growth period of the mixed culture, and that possibly the hybrid cells were present in a frequency higher than that reported. These opinions can not be supported with experimental results since the above mentioned technical difficulties hampered analysis of the mixed culture during its entire growth period.

It is difficult to explain the significant increase in the frequency of polyploid cells which occurred when the S-180 cells were transferred from the monolayer culture to the spinner culture. The only apparent change in environmental condition was the mechanical

mixing produced by the spinner apparatus. The increase in polyploid cells appears to be associated with an increase in the frequency of occurrence of endomitosis and possibly endoreduplication. The majority of the polyploid cells observed in the preparations made from S-180 cells grown in spinner culture were either in endometaphase or metaphase resulting from endoreduplication. The possibility exists that mechanical agitation triggers either one of these two mechanisms.

There was no significant difference between the frequency of binucleate cells found in the two separate cultures (results from the S-180 culture combined with results from the L-5178Y culture) and in the mixed culture. These results may be interpreted in one or both of two ways: (1) The presence of binucleate cells in a cell population is in no way related to a process of cell hybridization occurring in that population. If such were the case then no significant difference would be expected in the frequency of binucleate cells found in the separate cultures and the mixed culture. The binucleate cells present in all cases would be the result of some mechanism other than cell fusion. (2) If, however, it is assumed that the binucleate cell represents an intermediate step in the process of cell fusion or cell hybridization (a not unreasonable assumption in the light of available evidence) two possibilities are evident: A) cell fusion or somatic cell hybridization occurs only between cells of different cell lines (i.e. inter cell line fusion). Then the frequency of binucleate cells should be significantly higher in the mixed culture than in the separate cultures or, B) cell fusion or somatic cell hybridization occurs between cells of the same line (intra cell line fusion) as well as between cells from different lines. In this case

there should be no difference in the frequency of binucleate cells found in the mixed and separate cultures.

The author believes that the results obtained in this experiment (although not providing evidence in favour) are consistent with the idea that somatic cell hybridization may occur between cells of the same cell line as well as between cells from different lines. It is possible that somatic cell hybrids are present in both of the separate cultures as well as the mixed culture. If this were the case, in the present experiment it would be impossible to distinguish intra cell line hybrids from polyploid cells produced by other mechanisms.

It is possible that somatic cell hybridization or cell fusion is a process which occurs at a low frequency in all cell populations. The mechanism which would induce the initial cell fusion is obscure, but it would probably involve some change or changes in the cytoplasmic membrane which would allow the cells to fuse.[68] Fusion would not necessarily be limited to two cells, it might involve several or many cells, the fusion of which would produce one multinucleate cell or one large polyploid cell. There is no doubt that in any cell population heteroploid cells with increased chromosome numbers could be produced by any of the mechanisms which originate with one uninucleate cell. The operation of these mechanisms within a cell population does not in any way exclude the possibility that

heteroploid cells with an increased chromosome number are produced in that same cell population by cell hybridization.

From an evolutionary point of view, somatic cell hybridization would be a more significant mechanism than any other which would result in the production of heteroploid cells with an increased chromosome number. Endomitosis or endoreduplication, c-mitosis, failure of cytokinesis to occur, and teloreduplication would produce heteroploid cells with an increased number of the same chromosomes which were present in the original diploid set. The resulting cell would gain flexibility because any subsequent loss of a chromosome, or structural change in a chromosome would be buffered by the presence of the additional identical chromosomes. However, an increase in chromosome number by cell hybridization would have the distinct advantage of providing genetic variation in the newly formed heteroploid cell. The hybrid cell would possess a combination of all the genetic changes that may have occurred during its formation and the growth of both of the parental cells (e.g., translocation, inversion, deletion, mutation, change in chromosome number). The new genetic combination might be an unfavorable combination and result in the reduced growth rate of the cell. On the other hand the new genetic combination might give the hybrid cell a selective advantage over the parental cells under existing or changing environmental conditions.

Assuming hybridization may possibly occur at a very low level in normal cell populations it is reasonable to suppose that: (1) The resulting hybrid cells would show no selective advantage under normal <u>in vivo</u> conditions since the diploid cell is strictly

regulated and highly adapted to the environmental conditions under which it exists and, (2) If some change were to occur in the normal diploid cells, or in the environmental conditions under which they exist (i.e., a change due to carcinogenesis, or a change resulting from the transfer of a cell population from an *in vivo* to an *in vitro* condition) then, it is possible that some genetic variation possessed by a hybrid cell might confer a selective advantage upon that cell. Such selective advantage would give rise to the proliferation of the hybrid cell in the cell population to the extent that in time the hybrid cell would constitute the majority. Such an occurrence is consistent with the events often observed when a neoplasm arises or when a primary cell population is transformed *in vitro* to a permanent cell line. As mentioned earlier, in both cases a diploid-heteroploid shift in chromosome number is often involved and the new cell population may exhibit chromosome morphology and phenotypic characteristics not evident in the original population.

Once formed, neoplastic cell populations undergo continuous change. It is possible that somatic cell hybridization plays a significant role in this change, and in the subsequent evolution of the tumor. Three pathways could be envisaged whereby somatic cell hybridization might allow for the change in the characteristics of a neoplastic cell population: (1) fusion of neoplastic cells with other neoplastic cells of the same type, (2) fusion of neoplastic cells with surrounding diploid cells and, (3) fusion of

one type of neoplastic cell with another type of neoplastic cell.

Tumor evolution would result from the first pathway only if the two neoplastic cells which fused were significantly variant and the resulting variation gave the heteroploid cell a selective advantage. The work discussed in the literature review by Defendi, and by Fedoroff suggests that the second pathway may in fact be important in tumor progression. Support for the third suggested pathway may come from analysis of events occurring in human carcinoma of the thyroid. There are several types of malignant lesions that occur in this gland. Most are reasonably slow growing. Two histologic types in particular - papillary carcinoma and follicular carcinoma, can exist together in the same gland, each growing at a slow rate. Occasionally for no apparent reason, many giant cells appear in the tumor, the growth rate becomes very rapid and within approximately six months the condition is terminal.[82] It is possible that these giant cells are the result of somatic cell hybridization. The hybrid cell, due to the fact that it is able to draw from the combined characteristics of both parental cell types, may have gained a selective advantage over both parental lines and therefore outgrew them at a very rapid rate.

The final steps of this project, if successful, will provide support for the hypothesis that tumor progression <u>in vivo</u> may involve somatic cell hybridization between different types of neoplastic cells.

SUMMARY AND CONCLUSIONS

Two malignant mouse cell lines (Sarcoma 180 (Foley), L-5178Y) were selected from a group of five available cell lines for growth in a mixed *in vitro* culture. The selection was made after consideration of the karyotype and growth requirements of each cell line. Observation of the separate and mixed cultures of L-5178Y and S-180 has permitted the following conclusions to be drawn:

1. Transfer of the Sarcoma 180 (Foley) cell line from a monolayer to a spinner culture resulted in an increased frequency of polyploid cells present. This four-fold increase in polyploid cells appeared to be due to an increase in the frequency of endomitosis and possibly endoreduplication.

2. The presence of binucleate cells in equal frequency in both the mixed and separate cultures of L-5178Y and Sarcoma 180 (Foley) is consistent with the hypothesis that somatic cell hybridization is a process which takes place at a low frequency in all cell populations.

3. After three weeks of incubation, hybrid cells appeared in a low frequency (4.5 per cent) in the mixed culture as evidenced by chromosomal analysis.

Initial attempts to grow the Sarcoma 180 (Foley) cell line and the L-5178Y cell line as solid tumors in C3H mice were successful. It appears that an *in vivo* combination of these cell lines is feasible,

but further studies on *in vivo* growth of each line are necessary before hybridization experiments can be carried out.

The research reported in this thesis forms a foundation for future *in vivo* hybridization studies which if successful, will provide support for the hypothesis that somatic cell hybridization is one of the mechanisms involved in tumor progression.

TABLE I

PLOIDY OF SARCOMA-180 CELLS IN MONOLAYER AND SPINNER CULTURE

	MONOLAYER	SPINNER
Number s Cells	92	66
Number 2s Cells	8	34
	100	100

$X^2 = 20.4$ $P < 0.005$

TABLE II

CHROMOSOME COUNTS IN OBSERVED HYBRID CELLS

CELL NO.	CHROMOSOME NO.	DESCRIPTION
1	168	s S-180 + 2s L-5178Y
2	165	s S-180 + 2s L-5178Y
3	160	s S-180 + 2s L-5178Y
4	132	s S-180 + 1s L-5178Y
5	124	s S-180 + 1s L-5178Y
6	121	s S-180 + 1s L-5178Y
7	118	s S-180 + 1s L-5178Y
8	105	s S-180 + 1s L-5178Y
9	103	s S-180 + 1s L-5178Y

TABLE III

BINUCLEATE CELLS IN SEPARATE AND MIXED CULTURES OF S-180 AND L-5178Y

CULTURE	NO. CELLS COUNTED	NO. BINUCLEATE CELLS
Mixed S-180 L-5178Y	300	14
S-180	150 } 300	7
L-5178Y	150 }	

$X^2 = 2.42$ $P > 0.10$

Figure 1. Spinner culture flask showing teflon-coated bar mounted on a swivel within the culture vessel.

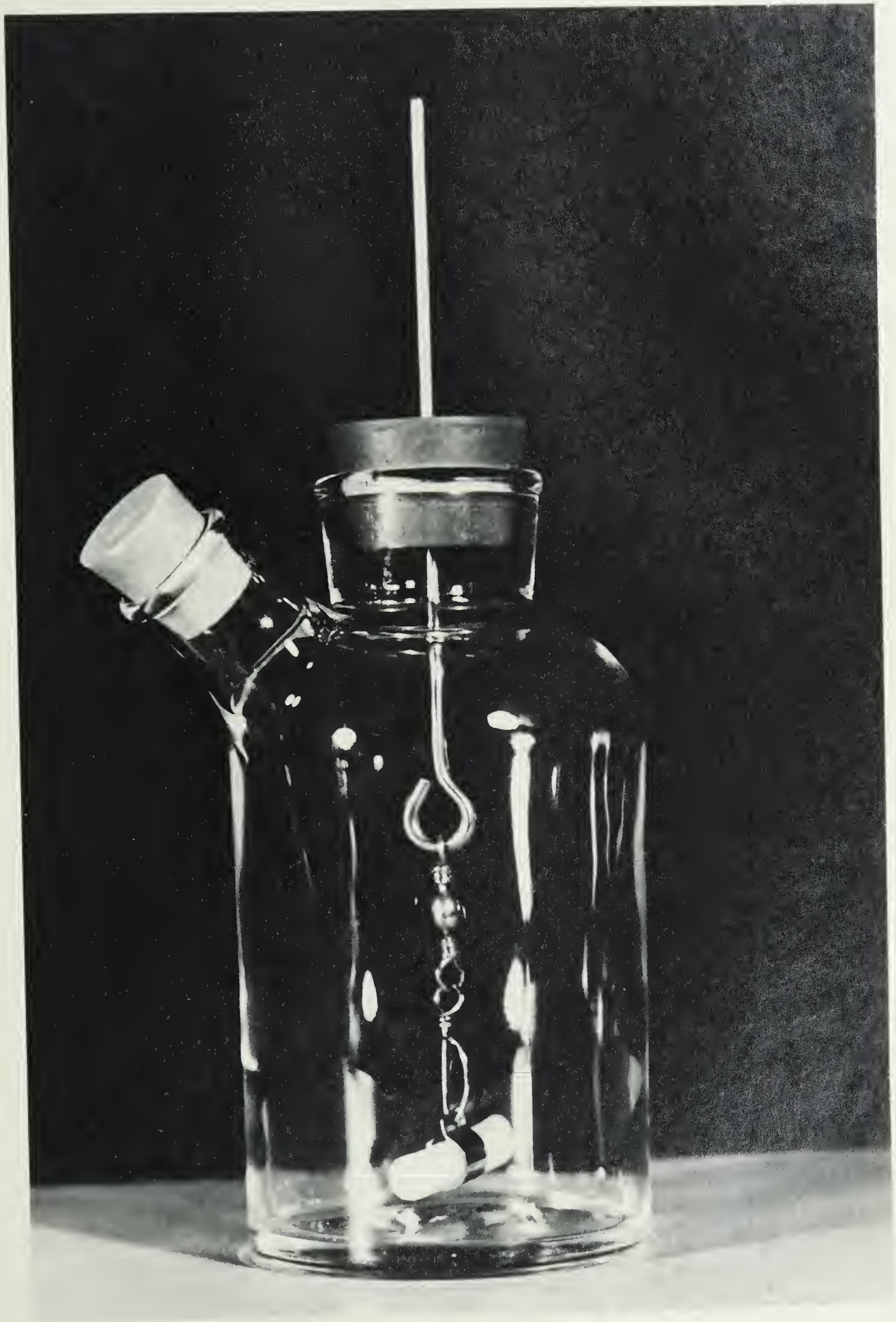


Figure 2. Representative karyotype of the Ehrlich ascites carcinoma (72 chromosomes).

Figure 3. Representative karyotype of the Strain L clone 929 cell line showing the D marker chromosome (67 chromosomes).

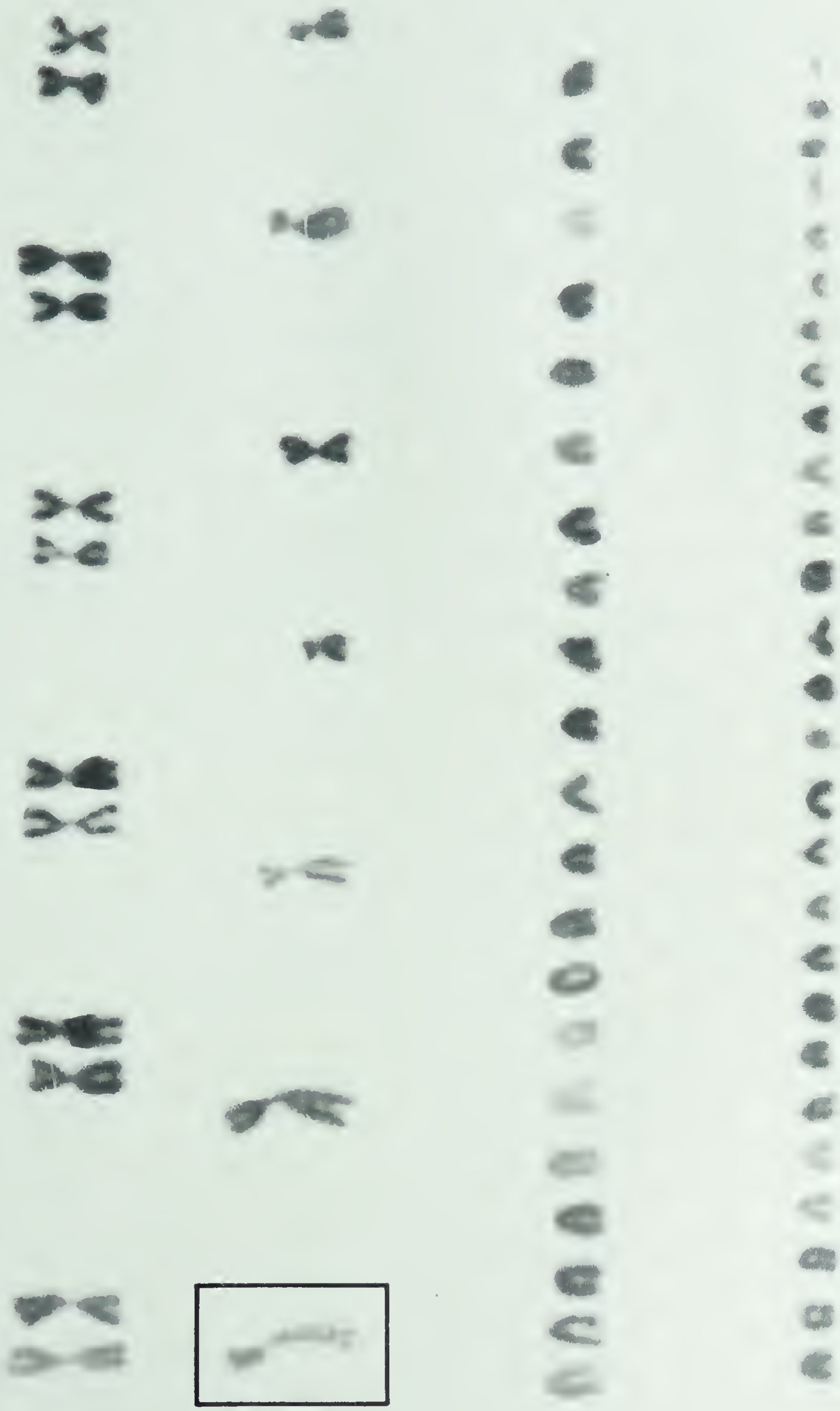


Figure 4. Representative karyotype of the Strain L clone 929 cell line showing the T marker chromosome (66 chromosomes).

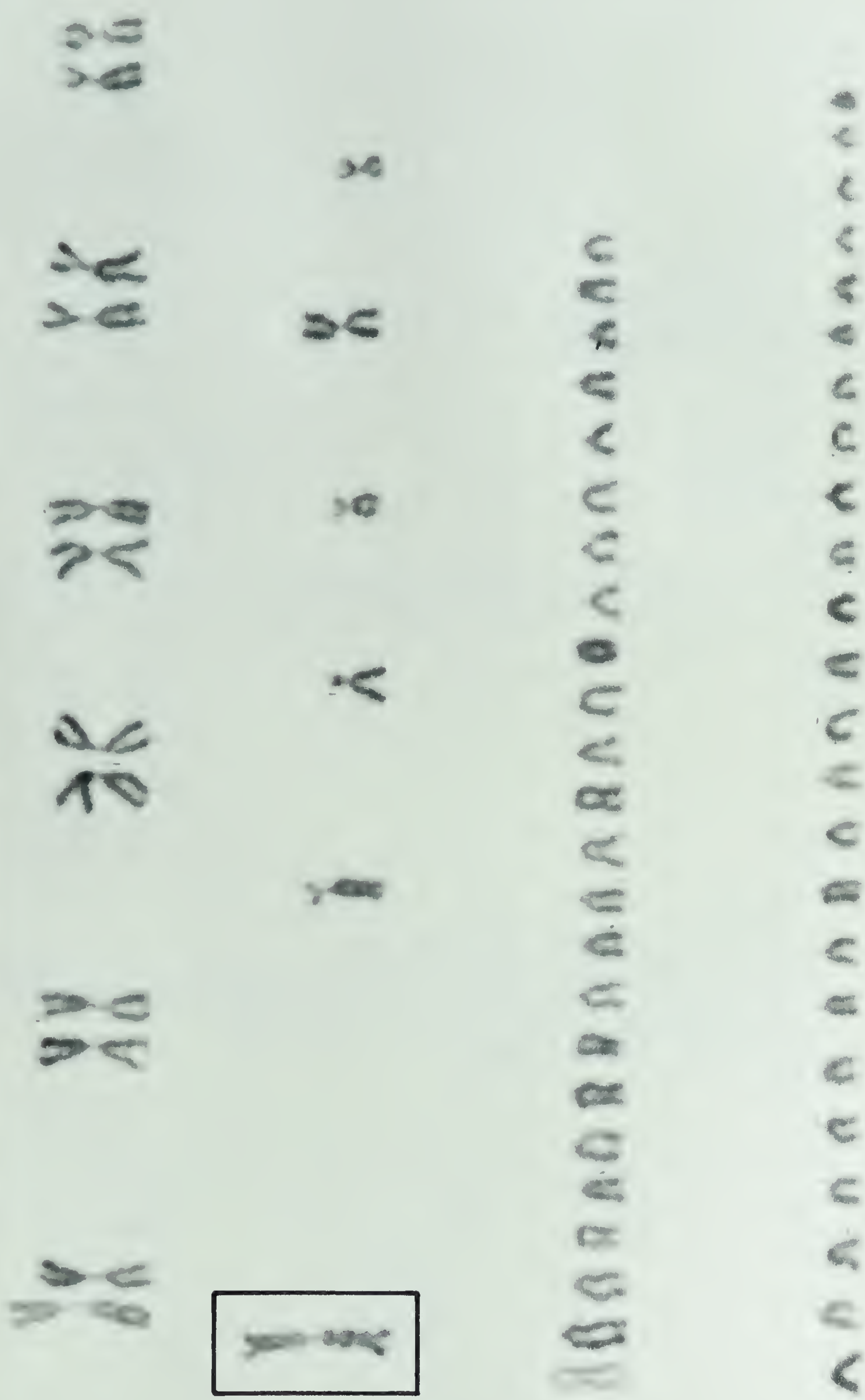


Figure 5. Histrogram showing the chromosome counts in 100 cells of L-5178Y mouse lymphoma cell line.

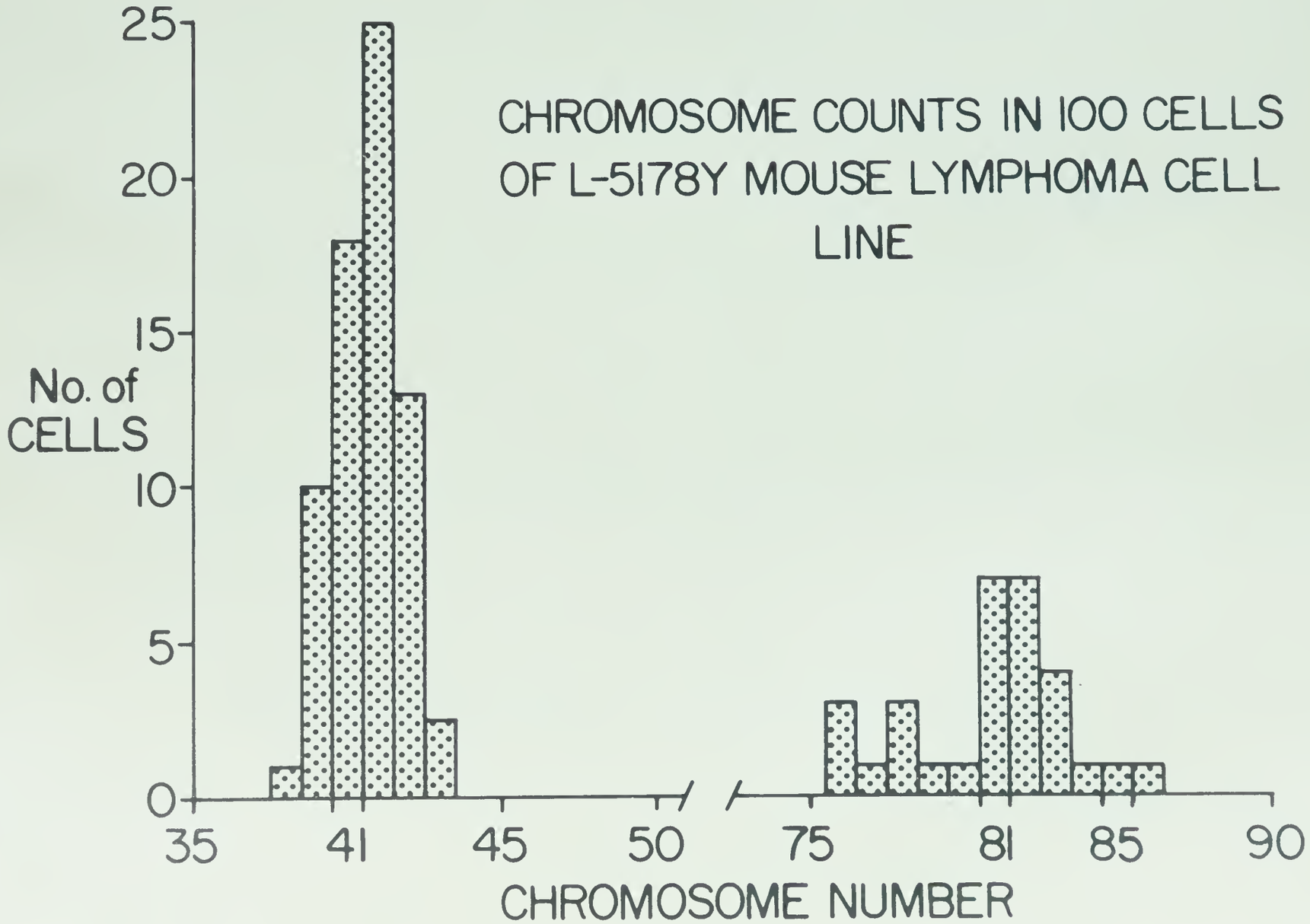

CHROMOSOME COUNTS IN 100 CELLS OF L-5178Y MOUSE LYMPHOMA CELL LINE
No. of CELLS
25
20
15
10
5
0
35
41
45
50
75
81
85
90
CHROMOSOME NUMBER

Figure 6. Representative karyotype of the L-5178Y mouse lymphoma cell line (41 chromosomes (s)).

Figure 7. Histrogram showing the chromosome counts in 100 stemline cells of the Sarcoma 180 (Foley) cell line.

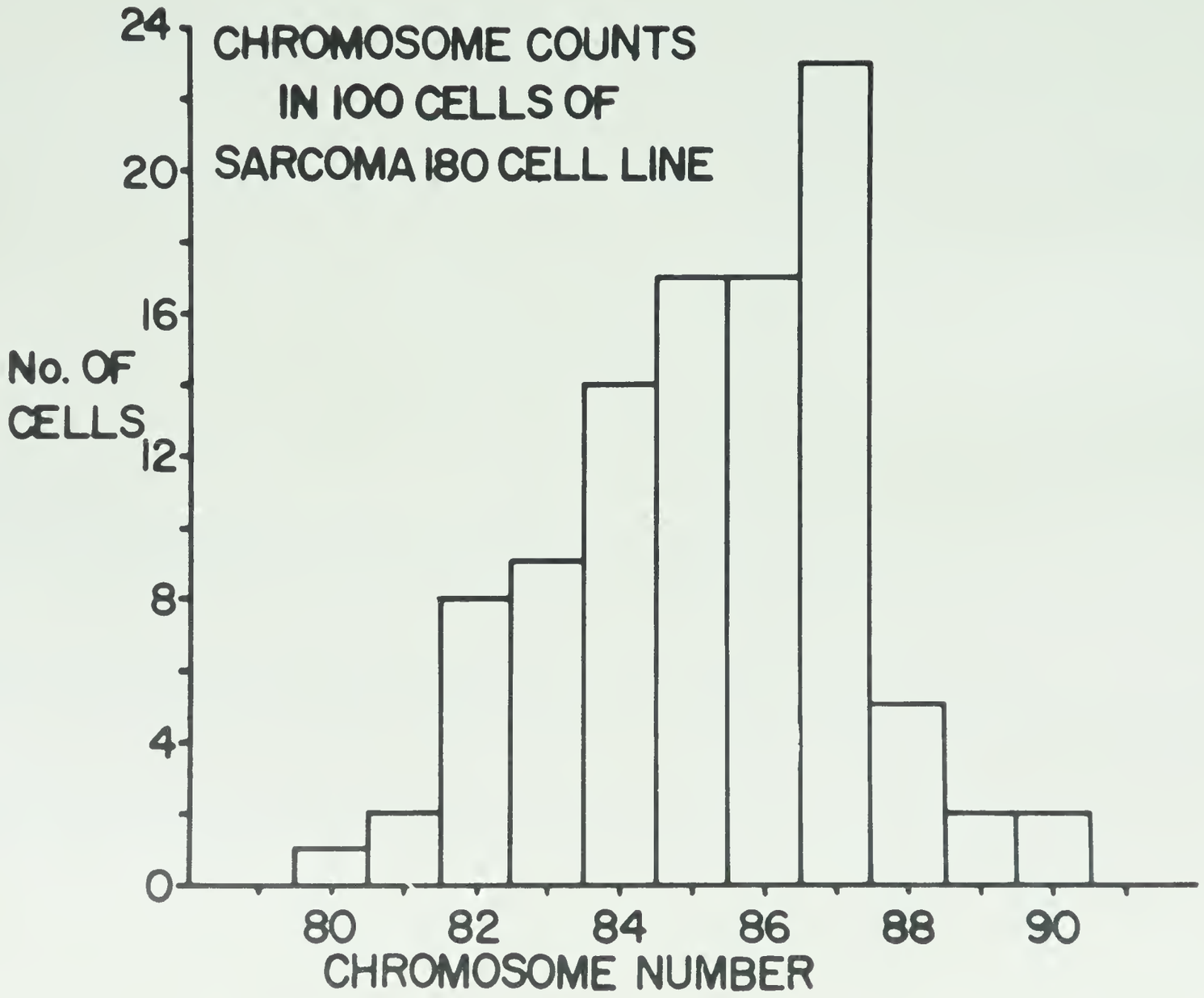

CHROMOSOME COUNTS
IN 100 CELLS OF
SARCOMA 180 CELL LINE
No. OF
CELLS
0
4
8
12
16
20
24
80
82
84
86
88
90
CHROMOSOME NUMBER

Figure 8. Representative karyotype of Sarcoma 180 (Foley) cell line (84 chromosomes (s)).

GROUP I
GROUP II
GROUP III

Figure 9. Suggested mechanism of origin of the metacentric chromosomes present in Sarcoma 180 (Foley) cell line. A reciprocal translocation between a telocentric and an acrocentric chromosome resulting in the formation of a metacentric and a minute chromosome.

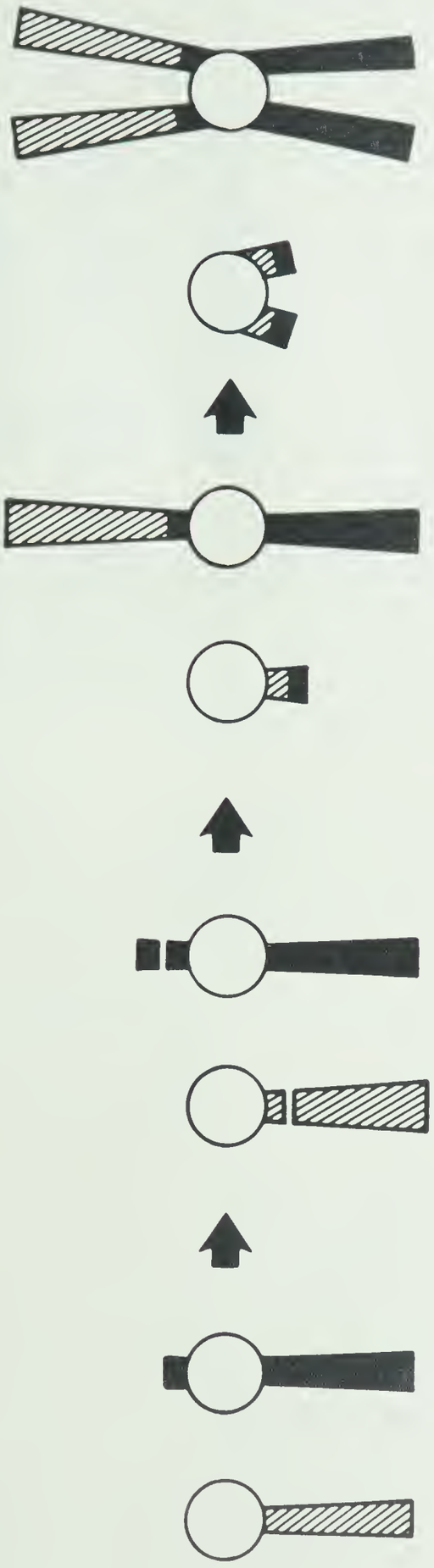


Figure 10. Histogram illustrating the results obtained upon chromosomal analysis of the mixed culture of L-5178Y and S-180 cell lines. Two hundred metaphase cells were analyzed, 57 cells were s L-5178Y; 119 were s S-180; four were 2s L-5178Y; 11 were 2s S-180. Nine cells were identified as hybrid (L-5178Y + S-180) cells.

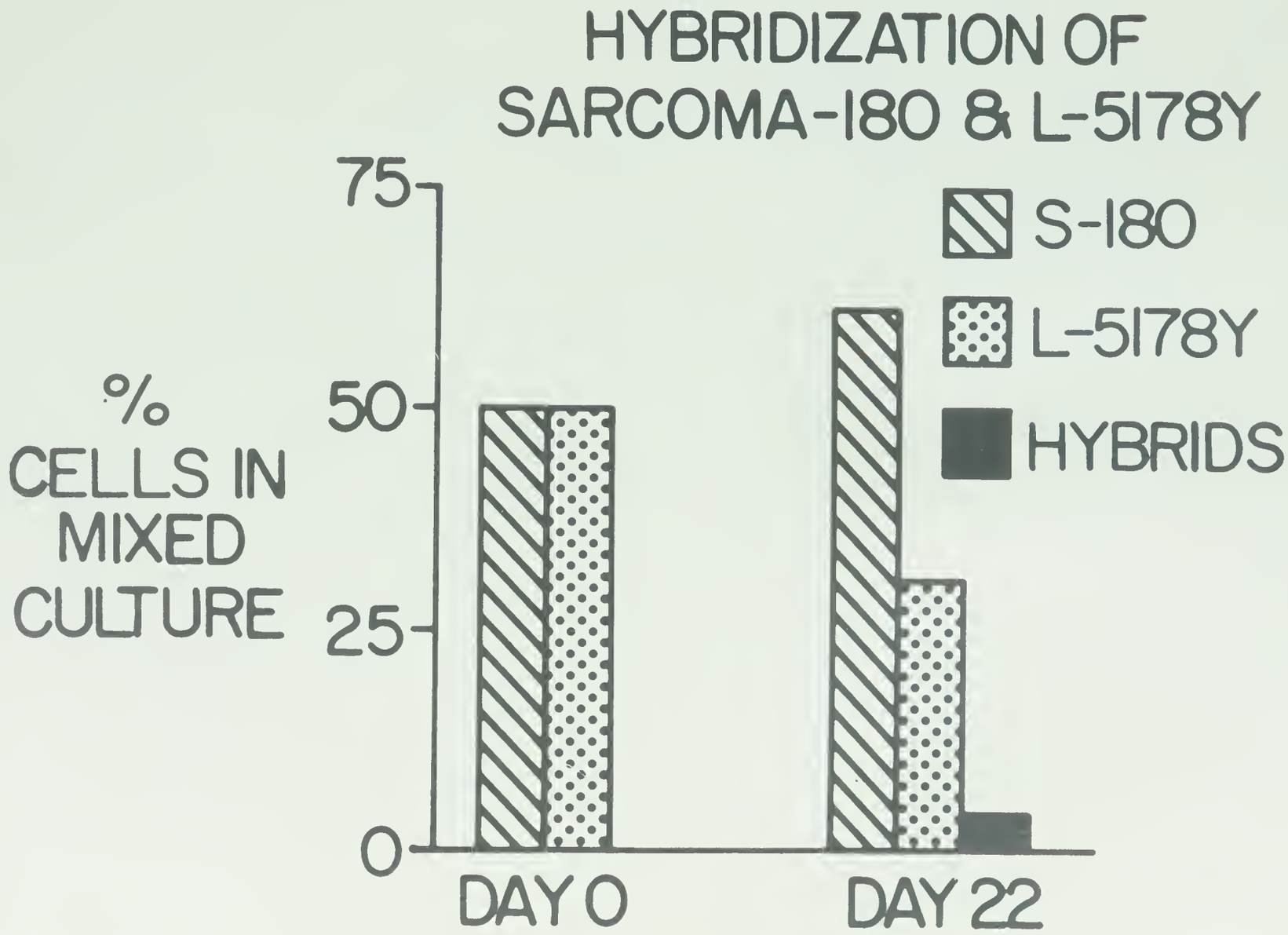
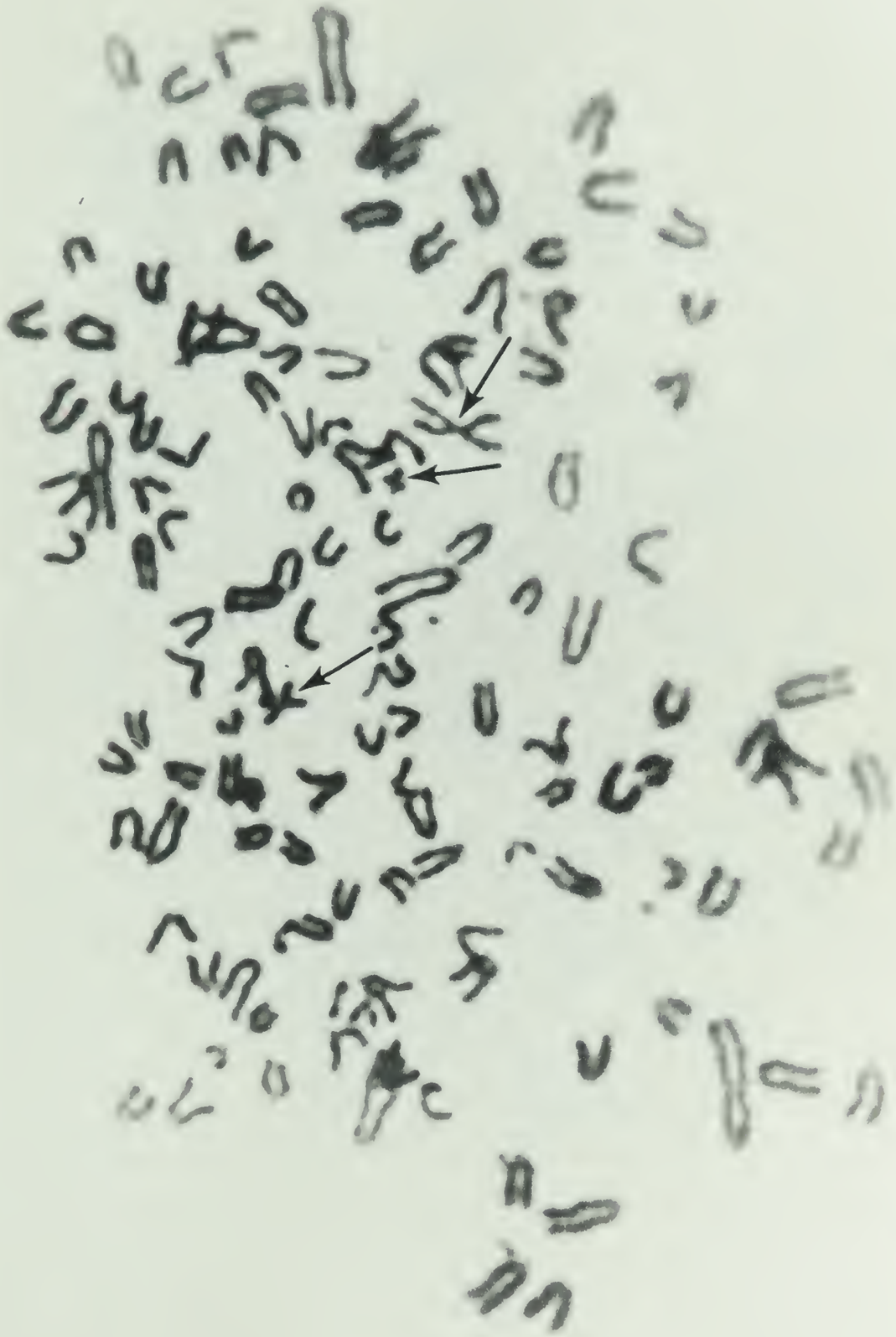
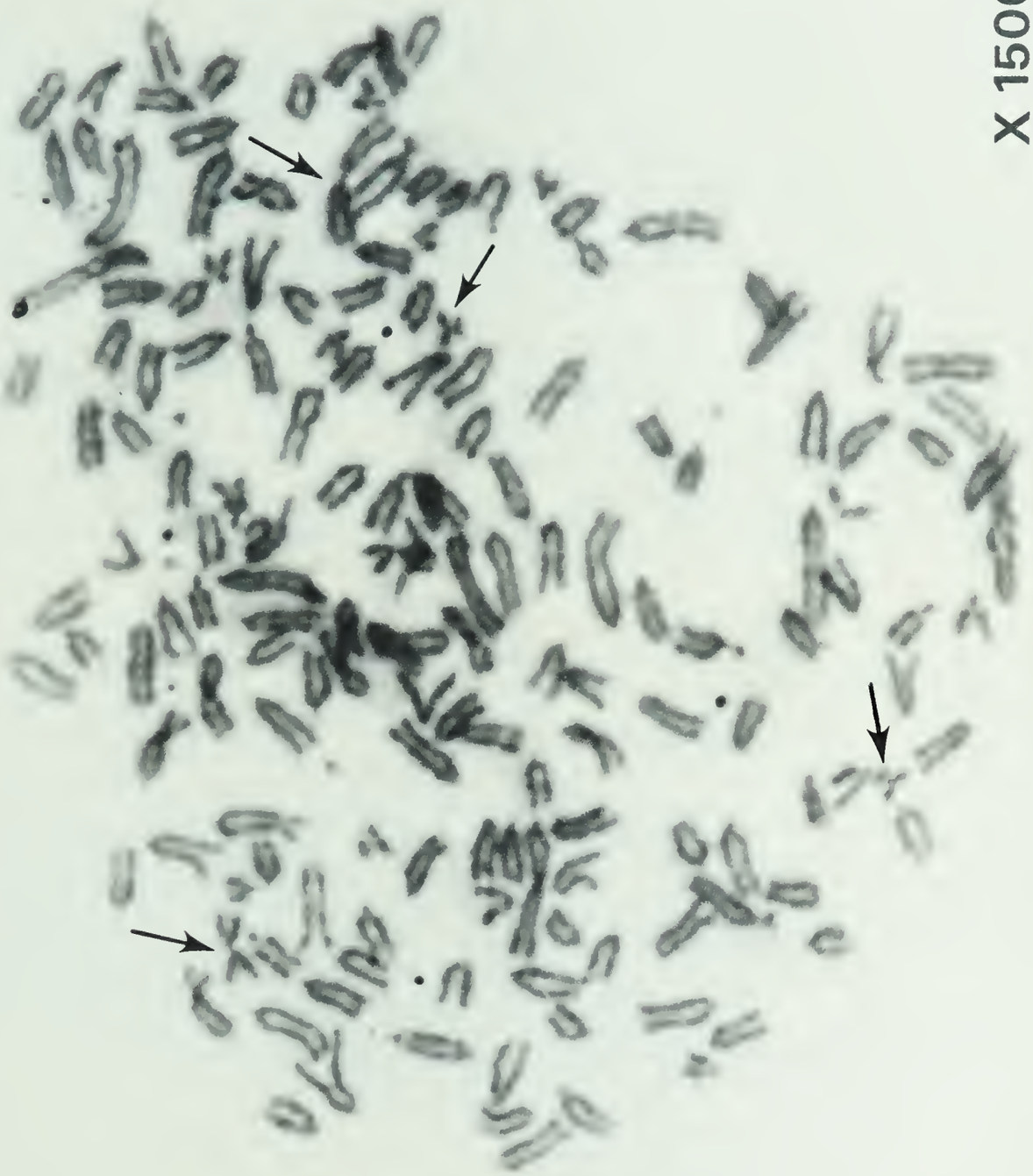

HYBRIDIZATION OF
SARCOMA-180 & L-5178Y
S-180
L-5178Y
HYBRIDS
% CELLS IN MIXED CULTURE
75
50
25
0
DAY 0
DAY 22

Figure 11. Hybrid cell with three metacentric marker chromosomes (large, medium-sized and small) and a chromosome number of 132 (s S-180 + s L-5178Y).



X 1650

Figure 12. Hybrid cell with four metacentric marker chromosomes (one large, one medium-sized and two small) and a chromosome number of 168 (s S-180 + 2s L-5178Y).



X 1500

Figure 13. Seven day old solid tumor of L-5178Y mouse lymphoma in the hind limb of a C3H mouse.

Figure 14. Seven day old solid tumor of Sarcoma 180 (Foley) in the hind limb of a C3H mouse.

Figure 15. Binucleate cell in mixed culture of Sarcoma 180 (Foley) and L-5178Y.

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